Functional Ecology 2007
21, 282–290

Scaling of insect metabolic rate is inconsistent with the nutrient supply network model

S. L. CHOWN,*† E. MARAIS,* J. S. TERBLANCHE,* C. J. KLOK,***
J. R. B. LIGHTON‡§ and T. M. BLACKBURN¶

**Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa; ‡Department of Biological Sciences, University of Nevada, Las Vegas, NV 89154, USA; §Sable Systems International, 7231 S Eastern Avenue B199, Las Vegas, NV 89119, USA; and ¶School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

Summary

1. The nutrient supply network model of the metabolic theory of ecology predicts that metabolic rate scales as mass$^{0.75}$ at all hierarchical levels.
2. An alternative, cell size, model suggests that the scaling of metabolic rate is a by-product of the way in which body size changes, by cell size or number, or some combination thereof. It predicts a scaling exponent of mass$^{0.75}$ at the widest interspecific level, but values of mass$^{0.67–1.0}$ for lower taxonomic groups or within species.
3. Here these predictions are tested in insects using 391 species for the interspecific analysis, and the size-polymorphic workers of eight ant species at the intraspecific level. In the latter, the contribution of ommatidium size and number to variation in body length, which is closely related to eye size, is used to assess the relative contributions of changes in cell size and number to variation in body size.
4. Before controlling for phylogeny, metabolic rate scaled interspecifically as mass$^{0.82}$. Following phylogenetic correction, metabolic rate scaled as mass$^{0.75}$.
5. By contrast, the intraspecific scaling exponents varied from 0·67 to 1·0. Moreover, in the species where metabolic rate scaled as mass$^{1.0}$, cell size did not contribute significantly to models of body size variation, only cell number was significant. Where the scaling exponent was < 1·0, cell size played an increasingly important role in accounting for size variation.
6. Data for one of the largest groups of organisms on earth are therefore inconsistent with the nutrient supply network model, but provide support for the cell size alternative.

Key-words: insects, intraspecific scaling, interspecific scaling, metabolic rate, metabolic theory of ecology.

Functional Ecology (2007) **21**, 282–290
doi: 10.1111/j.1365-2435.2007.01245.x

Introduction

How and why metabolic rate scales with body mass have implications for every level of biology (Damuth 1981; Suarez, Darveau & Childress 2004; Whitfield 2004; Gillooly *et al*. 2005a,b; Glazier 2005), and are consequently controversial. One of the most widely discussed models proposes that scaling is a consequence of the way in which nutrients are supplied through space-filling fractal networks, and predicts an exponent of 0·75, from molecules to organisms (West, Brown & Enquist 1997, 1999; West, Woodruff & Brown 2002). It forms the basis of the metabolic theory of ecology, which claims to explain much of biodiversity (Brown *et al*. 2004; Allen *et al*. 2006), and has been explored in a variety of contexts (e.g. Belgrano *et al*. 2002; Ernest *et al*. 2003; Economo, Kerkhoff & Enquist 2005; Gillooly *et al*. 2005a,b).

Kozłowski, Konarzewksi & Gawelczyk (2003a,b) present an alternative body size optimization model for the scaling of metabolic rate with body mass. They argue that this scaling is a by-product of the way in which body size changes, via adjustments in cell size or number or some combination thereof, and is optimized by natural selection. Body size expansion exclusively via changes in cell number results in an isometric scaling of metabolic rate because size changes are mediated through larger numbers of the same units. By contrast, when size changes are effected solely through an increase

†Author to whom correspondence should be addressed.
E-mail: slchown@sun.ac.za
**Present address: School of Life Sciences, Arizona State University, Box 874501, Tempe, AZ 885287-4501, USA

in cell size, metabolic rate scales with an exponent of 0·67. This component of the model was anticipated by Davison (1955) who first proposed a relationship between the scaling exponent of metabolic rate and the way in which cell size and number contribute to changes in body size. However, unlike Davison (1955), Kozłowski *et al*. (2003b) then go on to show that as a consequence of differences in body size optimization among lineages, the interspecific scaling relationship is constrained to lie between 0·7 and 0·8. Therefore, this model predicts that metabolic rate scales as *c.* 0·75 at the widest interspecific level, but that scaling exponents will vary between 0·67 and 1·0 at lower levels. Importantly, this model is not concerned with intraspecific variation associated with growth (i.e. ontogenetic variation), which results in fundamentally different scaling relationships to those found for adult organisms (Brooks & Wiley 1988; see also discussion in Glazier 2005).

Despite the fact that the nutrient supply network model and the cell size model make very different predictions for intraspecific and interspecific scaling exponents, few empirical investigations have sought to test these predictions. Indeed, empirical tests of the models have largely been restricted to assessments of the interspecific scaling exponents found for mammals and birds (Dodds, Rothman & Weitz 2001; Kozłowski *et al*. 2003a,b; White & Seymour 2003, 2004, 2005; Savage *et al*. 2004), although earlier work had considered intraspecific scaling in a variety of organisms (reviewed in Heusner 1982; Hulbert & Else 2004; Glazier 2005). One reason why nonontogenetic intraspecific scaling is less commonly assessed than interspecific scaling is the often narrow body size range of adults within a given species (especially in mammals and birds), which typically makes assessment of the intraspecific relationship between size and metabolic rate problematic (Brown, Enquist & West 1997). Although some species show substantial body size variation, this is frequently the consequence of sexual size dimorphism (Dunning 1992; Silva & Downing 1995), which would confound investigations of size effects.

By contrast, some insects, such as various ants, bees and beetles (Feener, Lighton & Bartholomew 1988; Emlen & Nijhout 2000; Peat *et al*. 2005), show considerable variation in the adult stage, often of an order of magnitude or more, that is not associated with gender. In beetles, this size variation is regularly associated with threshold-based differences in mating strategies (Emlen & Nijhout 2000), which are also likely to influence standard metabolic rates (Reinhold 1999). However, in several species of ants and bees, workers, which are all females, show extensive size variation that is often not associated with pronounced threshold effects (Feener *et al*. 1988; Peat *et al*. 2005). Although some differentiation exists in the tasks the worker caste undertakes, many of these activities involve similar levels of energetic investment such that, at a given temperature, the predominant influence on metabolic rate is size (Feener *et al*. 1988). In consequence, size-polymorphic workers provide an ideal opportunity to test the different predictions of the nutrient supply and cell size models. Moreover, insects are gaining increasing importance in the debate over these models, perhaps not surprisingly given that this group includes 60% or more of all extant species. Both sets of proponents claim that empirical data from the insects, on the interspecific scaling of metabolic rates, and on the conservation (or lack thereof) of tracheal cross-sectional area, can be used to test their models (Kozłowski & Konarzewski 2004; Brown, West & Enquist 2005). However, although previous studies have examined scaling of insect metabolic rate in a variety of contexts (Lighton *et al*. 2001; Addo-Bediako, Chown & Gaston 2002; Niven & Scharlemann 2005), a consensus interspecific scaling relationship for insects, based on a broad range of species from many higher taxa, which controls for phylogenetic nonindependence (Martins & Hansen 1997; Garland, Bennett & Rezende 2005) is not available. Here, we use metabolic rate data from eight polymorphic ant species (representing six genera and three subfamilies), the formicids as a family, and the insects as a whole to investigate intra- and interspecific scaling as a test of the predictions of the nutrient supply network and cell size models.

Materials and methods

INTRASPECIFIC DATA

To obtain the intraspecific data, whole colonies of ants were collected to minimize stress, and held under laboratory conditions for no more than a few weeks. Before respirometry, individual ants were starved for 1 h on moist filter paper because extended starvation can result in metabolic down-regulation (Lighton 1989). Each ant was weighed, and placed into a cuvette, which was placed within a Sable Systems AD-1 activity detector, and left to settle for 20–30 min before respirometry. Respirometry continued for one or more hours depending on activity of the individual, after which the ant was weighed again. Air (21% O_2 and N_2 balance) was passed through sodalime and Drierite columns to remove CO_2 and H_2O. From there the clean air flowed at a fixed rate [corrected to standard temperature and pressure (STP) using mass flow controllers] through either a CO_2 infra-red gas analyser or an oxygen analyser, connected to a computer that recorded all instrument output simultaneously. The system was housed within a temperature controlled cabinet that regulated temperature between 20 and 30 °C depending on the species (see Appendix S1 of the Online Supplementary Material). Data were exported to Sable Systems Datacan V software, where VCO_2 or VO_2 readings (STP) for periods of minimal activity and low metabolic rate were used. Data that were indicative of activity or stress were discarded. Metabolic rates were converted to microwatts assuming joule equivalences of 24·65 kJ L^{-1} (CO_2) or 20·7 kJ L^{-1} (O_2). Because mass measurements have

typically much less variation than measurements of metabolic rate, ordinary least squares regression was used to assess the relationship between $\log_{10}$ mass and $\log_{10}$ metabolic rate (see McArdle 1988 for defence of the least squares approach under these conditions).

The major prediction of the model proposed by Kozłowski *et al*. (2003b) is that where body size increases are effected solely by increases in cell number, metabolic rate allometry should have an exponent of 1, and where size increases are a consequence solely of cell size increase the exponent should be 0·67. Therefore, we selected four species from across the full spectrum of exponents (*Messor capensis*, *Camponotus fulvopilosus*, *C. maculatus* and *Anoplolepis steinergroeveri*), and estimated the contribution of cell size and number to body size variation. Given the wide variety of scaling exponents we expected consistent differences in the contributions of cell size and number to body size in the direction predicted by Kozłowski *et al*. (2003b). Although we used body length as a proxy for body size, the two variables are very closely related (Kaspari & Weiser 1999) and the outcome of the analyses was identical where mass was used (in *Messor capensis*, see Results).

To determine the contribution of cell size and number to body length variation, a readily measurable group of cells was required. Clearly, variation among tissues and organs is likely to exist (Chapman 1998; Weiser & Kaspari 2006). Therefore, any tissue that is used should be considered an approximation of the contribution of cell size and number change to body size variation. Stevenson, Hill & Bryant (1995), Montagne *et al*. (1999) and Kramer *et al*. (2003) have demonstrated that in *Drosophila*, eye size is a useful proxy for overall body size, and one where the contributions of ommatidial size and number can be used to estimate the contribution of cell size and number to changes in overall body size (see also earlier work on crayfish by Davison 1956). In ants, eye size shows substantial variation and is strongly related to overall body size (Weiser & Kaspari 2006). Therefore, we used the compound eyes to determine the contribution of cell size and number to body length variation, recalling that the corneal lens of each ommatidium represents the product of two epidermal, cornegean cells (Chapman 1998).

Total body length was measured using a StereoLEICA MZ 7·5 (Leica Microsystems, Wetzlar, Germany) microscope, fitted with an ocular micrometer. Scanning electron microscopy was used to measure ommatidium size (eye size measurements are subject to error using light microscopy, see Weiser & Kaspari 2006), and to count the number of ommatidia. After total body length measurements were completed, individually coded and labelled ants were decapitated. The heads were dissected dorsoventrally and mounted on adhesive paper for Scanning Electron Microscopy (Leo 1430VP, Carl Zeiss, Oberkochen, Germany). Care was taken to ensure that ant eyes were mounted perpendicular to the electron beam to prevent optical distortion. After gold sputter-coating, vertical and oblique (to verify ommatidial counts) micrographs were obtained with a beam strength of 7 keV and stored in digital form. Corneal lenses of each ommatidium were easily distinguishable and were counted to obtain a proxy for cell number (hereafter cell number). The cells were hexagonal (Fig. 1) and were measured using Albion CAD software (Choice Computing Inc., Stellenbosch, South Africa; www.choicecomp.com) to obtain an estimate of cell size (hereafter cell size). The mean of at least five corneal lens areas (in μm^2) in the central part of the eye was used to represent cell size. Owing to differential abundance of ants and the difficulty of ensuring perpendicular imaging, sample sizes differed between species and ranged from 19 to 40 individuals per species for which complete data sets were available. In the case of *Messor capensis*, individuals were weighed, prior to measurement, to the nearest 0·1 mg using a Mettler

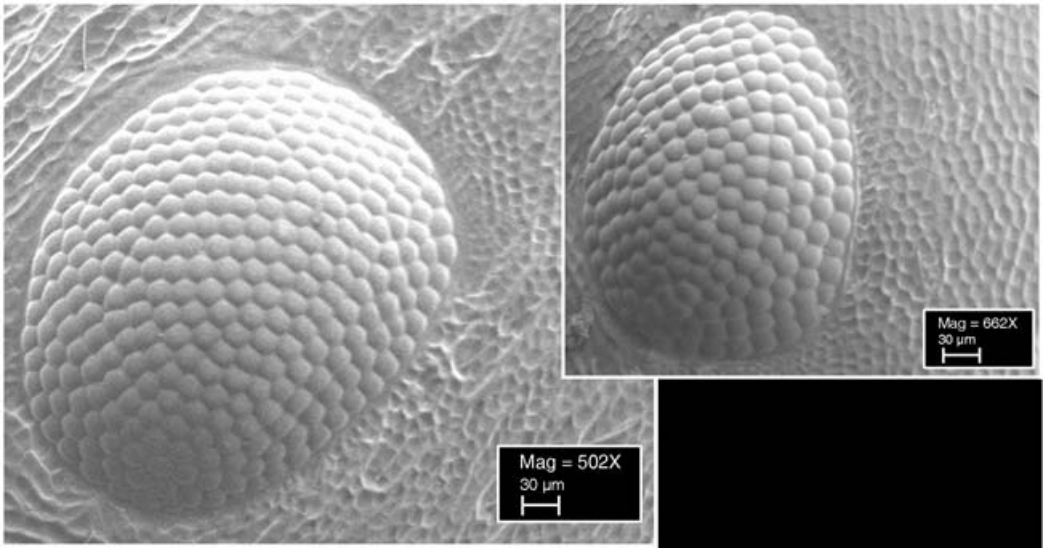

Fig. 1. Scanning electron micrographs of the compound eye of a large individual (9·8 mg, 7·56 mm) (left), and small individual (3·1 mg, 5·30 mm) (right) of *Messor capensis*. Note the similarity in cell size, but difference in cell number.

Toledo (Columbus, OH, USA) AX 504 microbalance. The contribution of variation in cell size and number to body size (length or mass) was estimated using a general linear model with body size as the dependent variable and a sums of squares approach that estimated the unique contribution of each of the independent variables to the variation in body size. This approach assumes that the measurement error in the independent variables was less than that of the dependent variable (see above), which initial investigations of repeatability suggested was reasonable.

INTERSPECIFIC DATA

Interspecific data were extracted from the literature and building on a previously unpublished data base (Addo-Bediako *et al.* 2002). Where any doubt existed regarding methods, quality of the data, or the activity state of the insects in question, the data were excluded. Multiple data for the same species were reduced to a single data point by a decision tree that selected flow-through methods first, then an experimental temperature closest to 25 °C, then any other real-time recording, and finally the most carefully described closed-system work. Data were provided in a variety of units and these were converted to microwatts assuming a Q10 of 2·0 (to a temperature of 25 °C) unless the original paper suggested a different value, and a respiratory quotient of 0·84 unless otherwise stated in the original work. For each species the measurement method was noted and simplified to a closed system or open system. The wing status (flying/nonflying) of each species was also noted (see Appendix S2 of the Online Supplementary Material). Ordinary least squares regression was used to establish the relationship between $\log_{10}$ mass and $\log_{10}$ metabolic rate for the insects as a whole, and independently for the Formicidae (including only workers). To investigate the likely effects of method and wing status on the scaling exponent for the insects as a whole, a general linear model with $\log_{10}$ metabolic rate as the dependent variable and $\log_{10}$ mass, wing status and respirometry method as the independent terms was constructed.

The analyses for the Insecta, and for the ants separately, were repeated to account for phylogenetic nonindependence using the method of phylogenetic generalized least squares (PGLS) (Grafen 1989; Martins & Hansen 1997). We compiled a supertree for this analysis using data from the Tree of Life (http://tolweb.org/tree/phylogeny.html) and from a wide variety of published phylogenies; it is available on request. We assumed all branches in this phylogeny were of equal length, although our conclusions are the same if the tree is assumed to be ultrametric with branch lengths scaled by taxon richness. PGLS explicitly incorporates the expected covariance among species into a statistical model fit by generalized least squares. The correlation between error terms, which is assumed to be zero in OLS, is thus altered in PGLS to reflect the degree of phylogenetic relatedness among the species. PGLS can be shown to be exactly equivalent to the widely used method of independent contrasts for a completely resolved phylogeny and the assumption that traits evolve by a 'Brownian motion' model of evolution (Rohlf 2001). However, the covariance matrix can be modified in PGLS to accommodate the degree to which trait evolution deviates from Brownian motion, using a measure of phylogenetic correlation, λ (Pagel 1999). λ normally varies between 0 (no phylogenetic correlation) and 1, with the value of λ thus specifying the extent to which trait evolution is phylogenetically correlated. We used a maximum likelihood approach to estimate optimal λ for each analysis. For Insecta as a whole, the value of λ was always $> 0{\cdot}85$, indicating strong phylogenetic correlation, and phylogenetic models were always markedly better fits to the data than nonphylogenetic models (ΔAIC > 100). Metabolic scaling exponents for Insecta thus must be interpreted in a phylogenetic context. By contrast, λ for ants alone was low ($< 0{\cdot}2$), indicating that phylogenetic control is not necessary, but phylogenetic and nonphylogenetic methods anyway produced near identical results.

Results

Within each of the ant species, body mass varied substantially (0·66–1·22 orders of magnitude, Appendix S1), and the slopes of the intraspecifc body mass–metabolic rate relationships were heterogeneous ($F_{7,228} = 4{\cdot}05$, $P = 0{\cdot}0003$). This heterogeneity disappeared following exclusion of *M. capensis* from the data ($F_{6,181} = 1{\cdot}06$, $P = 0{\cdot}386$). The lowest value did not differ significantly from the Euclidean geometric prediction of 0·67 and the highest value did not differ from an exponent of 1·0 (Table 1). Of the eight species, four had allometric exponents distinguishable from 0·75, while the other four did not.

In the species with the largest body mass–metabolic rate exponent, *Messor capensis* (*c.* 1), cell size did not contribute significantly to the increase in body size. Rather, only cell number was significant and it explained a substantial proportion (87%) of the variation in body length and body mass (Table 2). In the remaining species, which all had scaling exponents with 95% confidence intervals (CIs) either including 0·75, or below this value, both cell size and number contributed to the increase in body length. Judging by the *F*-values, the contribution of both independent variables increased in equivalency as the CIs declined away from inclusion of the exponents 1 and 0·75, though the CIs all included 0·67 in these species (but not in *M. capensis*). The substantial difference between *M. capensis* and the other species was also reflected in the scaling of metabolic rate given heterogeneity of slopes with its inclusion, but homogeneity when it was removed from the analysis.

For the ants as a whole, data were available for 43 species, and metabolic rate scaled as mass$^{0{\cdot}69}$ (95% CIs 0·58–0·81) in ordinary least squares regression, and as

Table 1. Intraspecific allometric exponents of the scaling of metabolic rate (μW) on mass (g) in eight ant species

Species	*n*	Size range	Slope ± SE	95% Confidence limits
Anoplolepis steinergroeveri	28	1·04	0·61 ± 0·082	0·44–0·78
Atta columbica	27	0·97	0·64 ± 0·095	0·44–0·84
Camponotus fulvopilosus	24	1·16	0·56 ± 0·077	0·40–0·71
Camponotus maculatus	50	1·22	0·60 ± 0·055	0·49–0·71
Eciton hamatum	25	1·06	0·84 ± 0·108	0·61–1·06
Formica rufa	20	0·66	0·69 ± 0·082	0·51–0·86
Messor capensis	49	1·09	1·28 ± 0·213	0·84–1·71
Messor pergandei	21	0·86	0·61 ± 0·062	0·48–0·74

Size range refers to the order of magnitude difference between the smallest and largest individuals measured.

Table 2. Outcome of the general linear models examining the contribution of cell size (μm^2) and cell number to body size (length (mm), except for *M. capensis* where mass (mg) is also included), in four of the eight ant species in which intraspecific scaling was examined. In each case the 95% CIs of the exponents of the body mass–metabolic rate relationships are also shown

Species	d.f.	MS	*F*	*P*	R^2
Messor capensis (exponent 95% CI 0·84–1·71)					
Mass					
Intercept	1	89·62	23·30	0·00002	0·870
Area	1	0·43	0·11	0·742	
Number	1	351·06	91·28	0·00001	
Error	37	3·846			
Length					
Intercept	1	89·62	23·30	0·00002	0·870
Area	1	0·422	0·11	0·742	
Number	1	351·07	91·28	0·00001	
Error	37	3·856			
Anoplolepis steinergroeveri (exponent 95% CI 0·44–0·78)					
Intercept	1	2·121	29·86	0·00001	0·962
Area	1	0·436	6·14	0·019	
Number	1	20·06	282·48	0·0001	
Error	31	0·071			
Camponotus maculatus (exponent 95% CI 0·49–0·71)					
Intercept	1	5·714	11·29	0·004	0·844
Area	1	5·056	9·99	0·0061	
Number	1	29·255	57·81	0·00001	
Error	16	0·506			
Camponotus fulvopilosus (exponent 95% CI 0·40–0·71)					
Intercept	1	0·307	0·46	0·504	0·415
Area	1	5·450	8·12	0·0077	
Number	1	6·695	9·98	0·0035	
Error	31	0·670			

$mass^{0·67}$ (95% CI 0·56–0·79) when accounting for phylogenetic nonindependence (PGLS; see Materials and methods). Neither value was distinguishable statistically from an exponent of either 0·75 or 0·67. Data for the Insecta included 391 species from 16 Orders (Appendix S2), and using an ordinary least squares model metabolic rate scaled as $mass^{0·82}$, which differed both from 0·67 and from 0·75 (Fig. 2). However, after accounting for phylogenetic nonindependence, metabolic rate scaled as $mass^{0·75}$, which differed from 0·67, but not from 0·75 (Fig. 2).

Previous investigations of the scaling of metabolic rate in insects, based on less comprehensive and phylogenetically nonindependent data, have demonstrated that wing status (flying or nonflying) and experimental method (closed or open system assessments) significantly influence metabolic rate and its estimation, respectively (Addo-Bediako *et al.* 2002). The inclusion of both wing status and experimental method with mass in a general linear model for metabolic rate resulted in a decline in the mass exponent to close to 0·75 (Table 3). The effect of wing status was significant in this model, but that of method was not. Additionally controlling for phylogeny using PGLS, the mass exponent was still close to 0·75, but now the effect of wing status was not significant whereas experimental method was (Table 3). The different result for wing status was probably largely due to the clustering of wingless species in the Coleoptera and Hymenoptera, such that when controlling for phylogenetic nonindependence the effect disappeared.

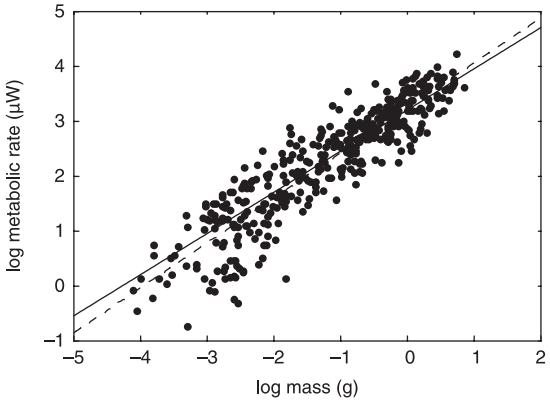

Fig. 2. Interspecific relationship between $\log_{10}$ mass (g) and $\log_{10}$ metabolic rate (μW) in insects. The data are adjusted to 25 °C (see Materials and methods) and are for 391 species from 16 orders. Ordinary least squares relationship (stippled line) described by the equation $\log_{10}$ metabolic rate = 3·26 + 0·82 × $\log_{10}$ mass. 95% confidence intervals for the mass exponent: 0·78–0·85. Relationship based on the phylogenetically independent contrasts (solid line), described by the equation $\log_{10}$ metabolic rate = 3·20 + 0·75 × $\log_{10}$ mass. Ninety-five per cent confidence intervals for the mass exponent: 0·70–0·79.

Discussion

Previous studies have investigated the scaling of insect metabolic rate in a variety of guises (reviewed in Chown & Nicolson 2004). However, they have typically used data that are far less comprehensive. Moreover, none have corrected for phylogenetic nonindependence (e.g. Lighton *et al*. 2001; Addo-Bediako *et al*. 2002), despite the importance of doing so from a theoretical perspective (Garland *et al*. 2005) and because λ is high, indicating that phylogenetic models provide a better fit to the data than nonphylogenetic models. Studies that have not corrected for phylogenetic nonindependence have typically found results quite different to those documented here. For example, Lighton *et al*. (2001) reported an exponent of 0·856, which, while close to the noncorrected value we found, differs substantially from 0·75. Likewise, Niven & Scharleman (2005) documented a slope of 0·66 based on a small (61 species), nonrepresentative data set. By contrast, the exponent of 0·75 for the interspecific allometric scaling of metabolic rate in insects documented here is neither a consequence of phylogenetic nonindependence, methodological problems (including small sample sizes), nor of interspecific differences in wing status.

The nutrient supply network model predicts that metabolic rate must scale as mass$^{0.75}$ at all hierarchical levels owing to three key properties of branching transport systems: space-filling networks, size-invariant terminal branch units, and minimization of energy expenditure in the networks by natural selection (West *et al*. 1997, 1999). Although this model allows for variation about the scaling exponent, it makes no specific predictions regarding this variation, except in the case of mammals (Savage *et al*. 2004), where the predictions are contentious (Kozłowski & Konarzewski 2004; White & Seymour 2004). Moreover, it suggests that metabolic rate should scale as mass$^{0.75}$ without accounting for phylogenetic effects (see, e.g. Savage *et al*. 2004). By contrast, the cell size model proposes that the way in which body size changes (cell volume or number) in the context of the size dependence of mortality and production rates determines the scaling of metabolic rate. If increases in size are mediated exclusively by changes in cell size then the scaling relationship should be mass$^{0.67}$, and if changes in size are due to increases in cell number the scaling relationship should be mass$^{1.0}$ (isometric) (Kozłowski *et al*. 2003b). Interspecific scaling values lie somewhere between these extremes as a consequence both of these processes and of body size optimization. Hence, this model predicts an interspecific scaling relationship of approximately 0·7–0·8 for phylogenetically diverse data sets, but does not constrain all metabolic rate scaling relationships to take this form.

Our results are consistent with the predictions of the cell size model. At the interspecific level in insects and in ants, metabolic rate scaled with an exponent of *c*. 0·7–0·8 depending on whether or not phylogeny was included. Without phylogenetic correction and at the broadest interspecific level, the scaling exponent differed significantly from 0·75, but not from 0·8. At the intraspecific level, the scaling of metabolic rate varied. Where body size increases were mediated exclusively by an increase in cell number, as in *Messor capensis*, the scaling of metabolic rate was isometric, or at least the scaling exponent was not statistically distinguishable from 1. In those cases where both cell size and number contributed to body size variation, the scaling exponents were less than 1. In addition, as the contribution of cell size and number became more equivalent, so the scaling exponent tended to decline.

None the less, variation about the scaling relationships meant that the CIs of these exponents overlapped substantially. Thus, our data did not allow us to dissect fully the nature of the relationship between cell size and number contributions to body mass variation and the metabolic rate–body mass relationship at this lower end of the spectrum of exponents. Clearly, there is additional scope for doing so. Whether similar results at the intraspecific level will hold for other groups of

Table 3. The effects of wing status and metabolic rate estimation method on the scaling of metabolic rate

	No phylogenetic control		Controlling for phylogeny	
Parameter	Estimate	Standard error	Estimate	Standard error
Intercept	2·94	0·054	3·11	0·179
Log mass	0·76	0·020	0·76	0·024
Wing status	0·38	0·048	0·08	0·060
Method	0·05	0·051	0·12	0·046

n = 347 for this analysis as data on wing status or experimental method are not available for all 391 species. Note that the simple allometric scaling models for metabolic rate give identical results using the full (n = 391) or reduced (n = 347) data sets, whether or not phylogeny is controlled for.

insects is not clear, but it is well established that in insects size differences can be mediated by a change in either cell size or number, or both depending on the species and the circumstance (Partridge & Coyne 1997; Huey *et al*. 2000).

Given that our results are consistent with the cell size model, the question remains whether they are inconsistent with the nutrient supply network model, and, by implication, the metabolic theory of ecology (Brown *et al*. 2004). This question is not straightforward. The nutrient supply network model does not make predictions for a relationship between the exponent of the body mass–metabolic rate relationship and the relative contributions of cell size and number to body size increase. Nor does the fundamental equation of the metabolic theory of ecology, which includes temperature effects on metabolic rate (Gillooly *et al*. 2001), predict consistent, directional variation in either the body mass–metabolic rate or temperature–metabolic rate relationships (with the exception of curvilinearity of the scaling of metabolic rates at the small end of the mammalian mass spectrum, see Kozłowski & Konarzewski 2005). However, the presence of variation about the relationships has been widely acknowledged and discussed in the context of the metabolic theory of ecology (e.g. Gillooly *et al*. 2001; Brown *et al*. 2004). Here, we found consistent, directional variation in the intraspecific scaling relationship of a form not predicted by the nutrient supply network model, suggesting that our findings are inconsistent with it. Moreover, although we found an interspecific scaling relationship that is identical to the prediction made by the nutrient supply network model (West *et al*. 1997), this was only the case following phylogenetic correction. The model's proponents make it clear that the effects of phylogeny are likely to be reflected in the constant (or intercept) but not the scaling exponent (Savage *et al*. 2004).

It therefore seems reasonable to conclude that our results support the cell size model (which also predicts an interspecific scaling exponent of *c*. 0·75) to the exclusion of the nutrient supply network model. None the less, we also recognize that additional, more sophisticated explorations of the nutrient supply network model and its alternatives (which include several others – reviewed in Glazier 2005) now need to be undertaken (see also Darveau *et al*. 2005a,b; Glazier 2005). Acknowledging that the nutrient supply network model's assumptions remain contentious (Dodds *et al*. 2001; Kozłowski & Konarzewski 2004, 2005; Suarez *et al*. 2004; Etienne, Apol & Olff 2006), it is clear that the most vigorous empirical debate concerns support for an exponent of 0·75 (White & Seymour 2003, 2004, 2005; Kozłowski & Konarzewski 2004; McKechnie & Wolf 2004; Savage *et al*. 2004; Brown *et al*. 2005; Glazier 2005; Hoppeler & Weibel 2005; Makarieva, Gorshkov & Li 2005; West & Brown 2005; Makarieva *et al*. 2006; White, Phillips & Seymour 2006). If many models make similar predictions for interspecific exponents, and if both measurement accuracy and laboratory conditions are likely to result in variation about the true relationship (McKechnie & Wolf 2004; Farrell-Gray & Gotelli 2005; McKechnie, Freckleton & Jetz 2006), then perhaps a closer inspection of both the mechanistic basis of the models, and the extent to which they make predictions for directional variation in relationships and/or the residual variance about them is also required (see also Glazier 2005). Similar arguments have been made both for the temperature component of the fundamental equation of the metabolic theory of ecology (Clarke 2006), and for one of ecology's other contentious models, the neutral model of biodiversity and biogeography (Ricklefs 2003; Gaston & Chown 2005; He 2005). Insects, and especially those groups in which considerable intra- and interspecific size variation are found, provide fertile ground for pursuing such expanded investigations, as we have demonstrated here.

Acknowledgements

We thank K.J. Gaston, R. Suarez and several anonymous referees for comments on the manuscript, though we do not necessarily agree with all of them. The South African National Research Foundation provided financial assistance.

References

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2002) Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology* **16**, 332–338.

Allen, A.P., Gillooly, J.F., Savage, V.M. & Brown, J.H. (2006) Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences of the USA* **103**, 9130–9135.

Belgrano, A., Allen, A.P., Enquist, B.J. & Gillooly, J.F. (2002) Allometric scaling of maximum population density: a common rule for marine phytoplankton and terrestrial plants. *Ecology Letters* **5**, 611–613.

Brooks, D.R. & Wiley, E.O. (1988) *Evolution as Entropy. Toward a Unified Theory of Biology*, 2nd edn. University of Chicago Press, Chicago.

Brown, J.H., Enquist, B.J. & West, G.B. (1997) Allometric scaling laws in biology. *Science* **278**, 373.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.

Brown, J.H., West, G.B. & Enquist, B.J. (2005) Yes, West, Brown and Enquist's model of allometric scaling is both mathematically correct and biologically relevant. *Functional Ecology* **19**, 735–738.

Chapman, R.F. (1998) *The Insects. Structure and Function*, 4th edn. Cambridge University Press, Cambridge.

Chown, S.L. & Nicolson, S.W. (2004) *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, Oxford.

Clarke, A. (2006) Temperature and the metabolic theory of ecology. *Functional Ecology* **20**, 405–412.

Damuth, J. (1981) Population density and body size in mammals. *Nature* **290**, 699–700.

Darveau, C.-A., Hochachka, P.W., Welch, K.C., Jr, Roubik, D.W. & Suarez, R.K. (2005a) Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. *Journal of Experimental Biology* **208**, 3581–3591.

Darveau, C.-A., Hochachka, P.W., Roubik, D.W. & Suarez, R.K. (2005b) Allometric scaling of flight energetics in orchid bees: evolution of flux capacities and flux rates. *Journal of Experimental Biology* **208**, 3593–3602.

Davison, J. (1955) Body weight, cell surface, and metabolic rate in anuran Amphibia. *Biological Bulletin* **109**, 407–419.

Davison, J. (1956) An analysis of cell growth and metabolism in the crayfish (*Procambarus alleni*). *Biological Bulletin* **110**, 264–273.

Dodds, P.S., Rothman, D.H. & Weitz, J.S. (2001) Re-examination of the '3/4-law' of metabolism. *Journal of Theoretical Biology* **209**, 9–27.

Dunning, J.B. (1992) *CRC Handbook of Avian Body Masses*. CRC Press, Boca Raton, FL.

Economo, E.P., Kerkhoff, A.J. & Enquist, B.J. (2005) Allometric growth, life-history invariants and population energetics. *Ecology Letters* **8**, 353–360.

Emlen, D.J. & Nijhout, H.F. (2000) The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* **45**, 661–708.

Ernest, S.K.M., Enquist, B.J., Brown, J.H., Charnov, E.L., Gillooly, J.F., Savage, V., White, E.P., Smith, F.A., Hadly, E.A., Haskell, J.P., Lyons, S.K., Maurer, B.A., Niklas, K.J. & Tiffney, B. (2003) Thermodynamic and metabolic effects on the scaling of production and population energy use. *Ecology Letters* **6**, 990–995.

Etienne, R.S., Apol, M.E.F. & Olff, H. (2006) Demystifying the West, Brown & Enquist model of the allometry of metabolism. *Functional Ecology* **20**, 394–399.

Farrell-Gray, C.C. & Gotelli, N.J. (2005) Allometric exponents support a 3/4-power scaling law. *Ecology* **86**, 2083–2087.

Feener, D.H., Lighton, J.R.B. & Bartholomew, G.A. (1988) Curvilinear allometry, energetics and foraging ecology: a comparison of leaf-cutting ants and army ants. *Functional Ecology* **2**, 509–520.

Garland, T., Bennett, A.F. & Rezende, E.L. (2005) Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology* **208**, 3015–3035.

Gaston, K.J. & Chown, S.L. (2005) Neutrality and the niche. *Functional Ecology* **19**, 1–6.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001) Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251.

Gillooly, J.F., Allen, A.P., West, G.B. & Brown, J.H. (2005a) The rate of DNA evolution: effects of body size and temperature on the molecular clock. *Proceedings of the National Academy of Sciences of the USA* **102**, 140–145.

Gillooly, J.F., Allen, A.P., Brown, J.H., Elser, J.J., Martinez del Rio, C., Savage, V.M., West, G.B., Woodruff, W.H. & Woods, H.A. (2005b) The metabolic basis of whole-organism RNA and phosphorus content. *Proceedings of the National Academy of Sciences of the USA* **102**, 11923–11927.

Glazier, D.S. (2005) Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews* **80**, 611–662.

Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society of London B* **326**, 119–157.

He, F. (2005) Deriving a neutral model of species abundance from fundamental mechanisms of population dynamics. *Functional Ecology* **19**, 187–193.

Heusner, A.A. (1982) Energy metabolism and body size. I. Is the 0·75 mass-exponent of Kleiber's equation a statistical artefact? *Respiratory Physiology* **48**, 13–95.

Hoppeler, H. & Weibel, E.R. (2005) Scaling functions to body size: theories and facts. *Journal of Experimental Biology* **208**, 1573–1574.

Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**, 308–309.

Hulbert, A.J. & Else, P.L. (2004) Basal metabolic rate: history, composition, regulation, and usefulness. *Physiological and Biochemical Zoology* **77**, 869–876.

Kaspari, M. & Weiser, M.D. (1999) The size-grain hypothesis and interspecific scaling in ants. *Functional Ecology* **13**, 530–538.

Kozłowski, J. & Konarzewski, M. (2004) Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant? *Functional Ecology* **18**, 283–289.

Kozłowski, J. & Konarzewski, M. (2005) West, Brown and Enquist's model of allometric scaling again: the same questions remain. *Functional Ecology* **19**, 739–743.

Kozłowski, J., Konarzewksi, M. & Gawelczyk, A.T. (2003a) Intraspecific body size optimization produces interspecific allometries. *Macroecology: Concepts and Consequences* (eds T.M. Blackburn & K.J. Gaston), pp. 299–320. Blackwell Science, Oxford.

Kozłowski, J., Konarzewski, M. & Gawelczyk, A.T. (2003b) Cell size as a link between noncoding DNA and metabolic rate scaling. *Proceedings of the National Academy of Sciences of the USA* **100**, 14080–14085.

Kramer, J.M., Davidge, J.T., Lockyer, J.M. & Staveley, B.E. (2003) Expression of *Drosophila* FOXO regulates growth and can phenocopy starvation. *BMC Developmental Biology* **3**, 5. http://www.biomedcentral.com/1471–213X/3/5.

Lighton, J.R.B. (1989) Individual and whole-colony respiration in an African formicine ant. *Functional Ecology* **3**, 523–530.

Lighton, J.R.B., Brownell, P.H., Joos, B. & Turner, R.J. (2001) Low metabolic rate in scorpions: implications for population biomass and cannibalism. *Journal of Experimental Biology* **204**, 607–613.

Makarieva, A.M., Gorshkov, V.G. & Li. B.-L. (2005) Biochemical universality of living matter and its metabolic implications. *Functional Ecology* **19**, 547–557.

Makarieva, A.M., Gorshkov, V.G., Li, B.L. & Chown, S.L. (2006) Size- and temperature-independence of minimum life-supporting metabolic rates. *Functional Ecology* **20**, 83–96.

Martins, E.P. & Hansen, T.H. (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* **149**, 646–667.

McArdle, B.H. (1988) The structural relationship: regression in biology. *Canadian Journal of Zoology* **66**, 2329–2339.

McKechnie, A.E. & Wolf, B.O. (2004) The allometry of avian basal metabolic rate: good predictions need good data. *Physiological and Biochemical Zoology* **77**, 502–521.

McKechnie, A.E., Freckleton, R.P. & Jetz, W. (2006) Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proceedings of the Royal Society of London B* **273**, 931–937.

Montagne, J., Stewart, M.J., Stocker, H., Hafen, E., Kozma, S.C. & Thomas, G. (1999) *Drosophila* S6 kinase: a regulator of cell size. *Science* **285**, 2126–2129.

Niven, J.E. & Scharlemann, J.P.W. (2005) Do insect metabolic rates at rest and during flight scale with body mass? *Biology Letters* **1**, 346–349.

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884.

Partridge, L. & Coyne, J.A. (1997) Bergmann's rule in ectotherms: is it adaptive? *Evolution* **51**, 632–635.

Peat, J., Darvill, B., Ellis, J. & Goulson, D. (2005) Effects of climate on intra- and inter-specific size variation in bumblebees. *Functional Ecology* **19**, 145–151.

Reinhold, K. (1999) Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* **13**, 217–224.

Ricklefs, R.E. (2003) A comment on Hubbell's zero-sum ecological drift model. *Oikos* **100**, 185–192.

Rohlf, F.J. (2001) Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* **55**, 2143–2160.

Savage, V.M., Gillooly, J.F., Woodruff, W.H., West, G.B., Allen, A.P., Enquist, B.J. & Brown, J.H. (2004) The predominance of Quater-power scaling in biology. *Functional Ecology* **18**, 257–282.

Silva, M. & Downing, J.A. (1995) *CRC Handbook of Mammalian Body Masses*. CRC Press, Boca Raton, FL.

Stevenson, R.D., Hill, M.F. & Bryant, P.J. (1995) Organ and cell allometry in Hawaiian *Drosophila*: how to make a big fly. *Proceedings of the Royal Society of London B* **259**, 105–110.

Suarez, R.K., Darveau, C.-A. & Childress, J.J. (2004) Metabolic scaling: a many-splendoured thing. *Comparative Biochemistry and Physiology B* **139**, 531–541.

Weiser, M.D. & Kaspari, M. (2006) Ecological morphospace of New World ants. *Ecological Entomology* **31**, 131–142.

West, G.B. & Brown, J.H. (2005) The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. *Journal of Experimental Biology* **208**, 1575–1592.

West, G.B., Brown, J.H. & Enquist, B.J. (1997) A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126.

West, G.B., Brown, J.H. & Enquist, B.J. (1999) The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science* **284**, 1677–1679.

West, G.B., Woodruff, W.H. & Brown, J.H. (2002) Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. *Proceedings of the National Academy of Sciences of the USA* **99**, 2473–2478.

White, C.R. & Seymour, R.S. (2003) Mammalian basal metabolic rate is proportional to body mass$^{2/3}$. *Proceedings of the National Academy of Sciences of the USA* **100**, 4046–4049.

White, C.R. & Seymour, R.S. (2004) Does basal metabolic rate contain a useful signal? Mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. *Physiological and Biochemical Zoology* **77**, 929–941.

White, C.R. & Seymour, R.S. (2005) Sample size and mass range effects on the allometric exponent of basal metabolic rate. *Comparative Biochemistry and Physiology A* **142**, 74–78.

White, C.R., Phillips, N.F. & Seymour, R.S. (2006) The scaling and temperature dependence of vertebrate metabolism. *Biology Letters* **2**, 125–127.

Whitfield, J. (2004) Ecology's big, hot idea. *Plos Biology* **2**, 2023–2027.

Received 18 September 2006; accepted 14 December 2006
Editor: Jan Kozłowski

Supplementary material

The following supplementary material is available as part of the online article (full text) from http://www.blackwell-synergy.com

Appendix S1. Metabolic rate data for eight species of size-polymorphic ants.

Appendix S2. Metabolic rates and body masses of insects used in this study.